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Biology of Young Winter Flounder *Pseudopleuronectes americanus* (Walbaum); Metabolism Under Simulated Estuarine Conditions[1,2]

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ABSTRACT

Metabolism based on laboratory studies of young winter flounder acclimated to estuarine-like conditions show that rates of oxygen uptake (ml O_2/hr/g), as a function in increasing temperature, do not differ significantly between salinities of 10 and 20 ‰. There is, however, a 40–50% decrease in the quantities of oxygen consumed by fish subjected to salinities of 10 and 20 ‰. Metabolic rates do not differ between fish sizes used (within the I+ group: 100–135 mm total length and 140–175 mm total length). Slopes in the equation, log metabolism = log a + b log weight, for temperatures of 24 C, 20 C, and 16 C (30 ‰ salinity) exceed unity.

INTRODUCTION

The effect of different salinities on the metabolism of estuarine fish has rarely been examined. Some investigators suggest estuarine fish possess metabolic mechanisms different from those species which reside in fresh and salt waters (Hoss, 1967; Kinne, 1964). Others report that euryhaline fish have increased metabolic rates at lower salinities (Schlieper, 1955; Job, 1969). Interpretation of these observations in terms of bioenergetics remains to be evaluated.

Winter flounder (Age-0 and I) remain within the influence of estuaries during their early developmental stages (Saila, 1961). Between Ages-I and II, specimens double in length (90 mm to 180 mm) and triple in weight (15 g–50 g) (Berry et al., 1965; Topp, 1967). Metabolic requirements during this period are perhaps greater than at any other time during the life of this species. The purpose of the present study, therefore, is to determine the respiration rate for juvenile winter flounder under simulated estuarine conditions.

MATERIALS AND METHODS

A continuous-flow, gravity-feed respirometer was designed from available apparatus (Fig. 1). A sea water reservoir placed 85 cm above the respiration chamber insured adequate head pressure. Sea water passed via glass tubing through a manually controlled tap-water bath (± 1.0 C) into an aeration chamber, and through B.O.D. 1, the respiration chamber, B.O.D. 2, and B.O.D. 3. Each B.O.D. bottle had a volume of 220 ml; the respiration chamber volume was 1350 ml. The entire system was insulted to provide constant temperature; hermetically sealed glass tubing insured constant sea water flow.

Temperatures were monitored at four sites with epoxy-coated copper-constantan thermocouples wired to a Leeds and Northrup[4] Potentiometer. I measured flow rates by collecting sea water in a tared glass vial for 1 minute and weighing the vial and contents on a Mettler H-5 pan balance.[4] Flow rates were controlled within the range 30–40 ml/min depending on the temperature and size of fish in the chamber. Each fish was permitted to extract 25–35% of the dissolved oxygen available. Differences in dissolved oxygen between B.O.D.'s 1 and 2 served as a measure of consumption by the fish.

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[4] The use of this instrument does not constitute an endorsement of this product by the National Marine Fisheries Service.

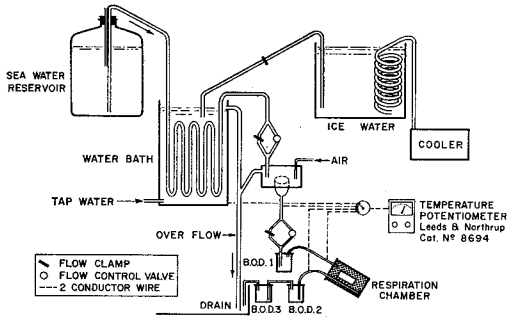

FIGURE 1.—Respirometer for measuring oxygen consumption in young winter flounder at different temperatures and salinities.

Twenty young winter flounder 100–135 mm total length (TL) were taken from the Weweantic River Estuary, Wareham, Massachusetts by otter trawl in June 1969; a second group (140–175 mm TL) was taken in August. All fish were transported to the laboratory in styrofoam coolers containing aerated artificial sea water of 30 ‰ salinity (Raila Marine Mix_R,[4] Teaneck, New Jersey). Cooler ice packs, sealed in polyethylene bags, were placed in the water to lower the temperature and reduce metabolic rates.

Ten flounder were placed in each of two 75-liter plexiglass aquaria containing sea water of 30 ‰ salinity. Fish were acclimated for 2 weeks at each of four temperatures (12 C, 16 C, 20 C, and 24 C) and two photoperiods, 13 hours light with the two lower temperatures and 15 hours light for the higher temperatures. Light intensity was approximately 885 lumens. Photoperiods were interpolated for 42° N. latitude from records of the U. S. Naval Observatory and temperatures were chosen from spring and summer values recorded in the Weweantic Estuary. A maintenance ration of chopped mussel, *Mytilus edulis*, was fed to the fish at 2-day intervals; fish selected for oxygen uptake determination were fasted for 24 hours prior to experiments. Aquaria were supplied with water filters packed with charcoal and sand, and the water was replaced weekly to protect against accumulation of waste products.

The dorsal and anal fins of each fish were crenellated to provide identification. Test specimens, identified by the fin marks, were randomly selected. Each specimen was placed in an open respiration chamber and submersed in the aquarium for 24 hours before the chamber was attached to the measuring apparatus.

To assess the variation among fish and to estimate the time required for metabolic rates to stabilize I conducted six trial tests on individuals ranging from 100–132 mm total length (10–24 g). All were acclimated to 12 C and 30 ‰ salinity. Oxygen consumption stabilized after 1 hour. Variability in results after 1½, 2 and 4½ hours was similar and was almost one-fourth that after 45 minutes.

Based on the results of the trial tests, the periodicity of tidal cycles in the Weweantic River estuary and subsequent changes in temperature and salinity (Frame, 1971), oxygen uptake (ml O_2/hr/g) was calculated for each fish at half-hour intervals following an initial 45-minute acclimating period. Test chamber salinities were lowered from 30 ‰ to 10 ‰ or 20 ‰ after 2¼ hours, and a second 45-minute acclimation period permitted the fish to adjust to new salinities before oxygen measurements were made at half-hour intervals. After 2¼ hours at the lower salinity, the fish were damp-dried and weighed. The total time elapsed under monitoring conditions amounted to 4½ hours per fish.

The routine metabolic rate (Fry, 1957) was measured by the unmodified Winkler method and was subjected to covariance analysis at selected levels in a factorial model (Steel and Torrie, 1960). The levels consisted of the following: four environmental conditions. two size categories (100–135 mm total length and 140–175 mm total length), and two salinities (10 ‰ and 20 ‰) (Table 1). All treatments were randomized and two observations comprised each cell, making a total of 32 possible observations. Data were analyzed with a BIOMD program adapted for use on the University Computing Center's C. D. C. 3600. Total metabolism was also calculated as a function of weight at three temperatures.

Oxygen consumption was converted to calories based on the assumption that 1.0 ml O_2 is equivalent to 5.0 calories (Swift and French, 1954; Paine, 1965; Paloheimo and Dickie, 1966).

TABLE 1.—*Experimental design for examining the respiration rates of young winter flounder*

	Fish size*	A_1 Salinity 20‰	A_2 Salinity 10‰
B_1	C_1	$a_1 b_1 c_1$	$a_2 b_1 c_1$
Early spring	C_2	$a_1 b_1 c_2$	$a_2 b_1 c_2$
12° C, 13 hr light			
B_2	C_1	$a_1 b_2 c_1$	$a_2 b_2 c_1$
Late spring	C_2	$a_1 b_2 c_2$	$a_2 b_2 c_2$
16° C, 13 hr light			
B_3	C_1	$a_1 b_3 c_1$	$a_2 b_3 c_1$
Early summer	C_2	$a_1 b_3 c_2$	$a_2 b_3 c_2$
20° C, 15 hr light			
B_4	C_1	$a_1 b_4 c_1$	$a_2 b_4 c_1$
Late summer	C_2	$a_1 b_4 c_2$	$a_2 b_4 c_2$
24° C, 15 hr light			

*C_1 = 100–135 mm, C_2 = 140–175 mm.

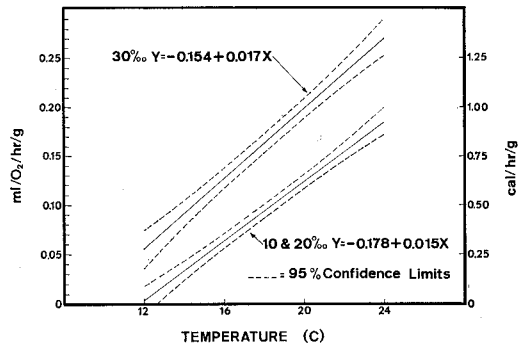

FIGURE 2.—Oxygen consumption for winter flounder at four temperatures and salinities of 30 ‰ and 10 ‰ and 20 ‰; Y = oxygen (ml O_2/hr/g), X = temperature (C).

RESULTS

At 30 ‰ salinity oxygen consumption varied linearly with increasing temperature (Table 2). Correlation coefficients were higher with the linear relationship than with semilogarithmic and other transformations. Differences in rates between small fish (100–135 mm total length) and large fish (140–175 mm total length) and between sexes were not significant (.05) for the two time periods. Since no significant differences in rates occurred between times, data were pooled to yield the following single common regression line for all flounder: $Y = -0.154 + 0.017X$ (Fig. 2), and for the exponential expression $Y = ab^X$: $Y = -4.449 + 0.155X$ (Fig. 3). Bartlett's test of homogeneity of variance applied to the values comprising the common line at the 2¼-hour time period indicated that homogene-

TABLE 2.—*Dissolved oxygen consumption values (ml O_2/hr/g) for 1+-year-old winter flounder for given temperatures, salinity changes, and times in respiration chamber*

	Time in chamber							
	30 ‰		10, 20 ‰					
Temperature	1 hr 45 min	2 hr 15 min	4 hr	4 hr 30 min	Weight (g)	Length (mm)	Salinity change	Sex
	0.3604	0.3570	0.2176	0.2066	35.48	154	20	Female
	0.3180	0.3108	0.1664	0.1554	56.88	175	20	Female
	0.2618	0.2758	0.2480	0.2400	42.50	161	10	Male
	0.2650	0.2568	0.1854	0.1924	55.97	163	10	Male
24 C	0.2916	0.3066	0.2416	0.2256	17.19	124	20	—
	0.2636	0.2614	0.1676	0.1416	14.96	115	20	—
	0.1860	0.1760	0.0810[1]	0.0956[1]	14.80	120	10	—
	0.2576	0.2556	0.0822[1]	0.0826	16.89	125	10	—
	0.1852	0.1904	0.1512	0.1498	54.06	171	20	Male
	0.1686	0.1694	0.0862	0.1062	32.63	154	10	Female
	0.2338	0.1882	0.0704	0.0684	27.41	141	10	Female
20 C	0.1642	0.1628	0.1048	0.1148	16.09	125	20	—
	0.1988	0.2066	0.1502	0.1334	17.92	128	20	—
	—	0.1896	0.0808	0.0792	17.46	125	10	—
	0.1968	0.1502	0.1554	0.1258	17.17	124	10	—
	0.1576	0.1436	0.0890	0.0750	55.03	175	20	Female
	0.1312	0.1142	0.0444	0.0390	39.48	160	20	Male
	0.1350	0.1514	—	0.0678	51.86	168	10	Male
16 C	0.0894	0.0988	0.0312	0.0516	34.12	152	10	Male
	0.1262	0.1050	—	0.0326	11.25	111	10	—
	0.0734	0.0588	0.0392	0.0476	19.11	124	20	Female
	0.0524	0.0400	0.0036	0.0034	48.66	167	20	Female
	0.0666	0.0430	0.0102	0.0024	26.15	141	20	Female
	0.0242	0.0216	0.0022	0.0112	30.18	150	10	Female
12 C	0.0344	0.0196	0.0238	0.0164	43.18	159	10	Male
	0.0568	0.1698	0.0216	0.0226	22.37	131	20	—
	0.0830	0.0802	0.0294	0.0232	18.90	125	20	—
	0.1114	0.1140	0.0086	0.0266	20.56	124	10	—

[1]Excluded from analysis.

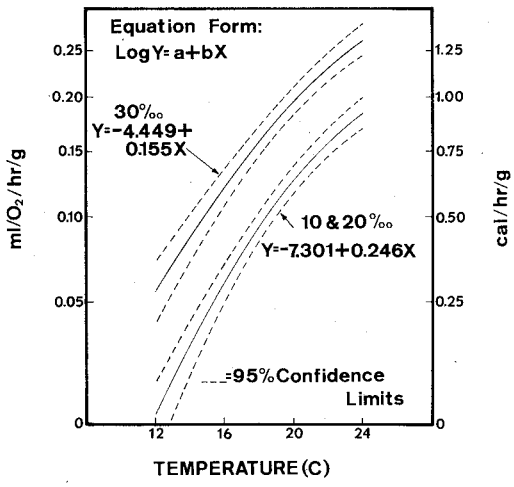

FIGURE 3.—Oxygen consumption for winter flounder at four temperatures and salinities of 30 ‰ and 10 ‰ and 20 ‰; semilogarithmic relationship; Y = oxygen (ml O_2/hr/g), X = temperature (C).

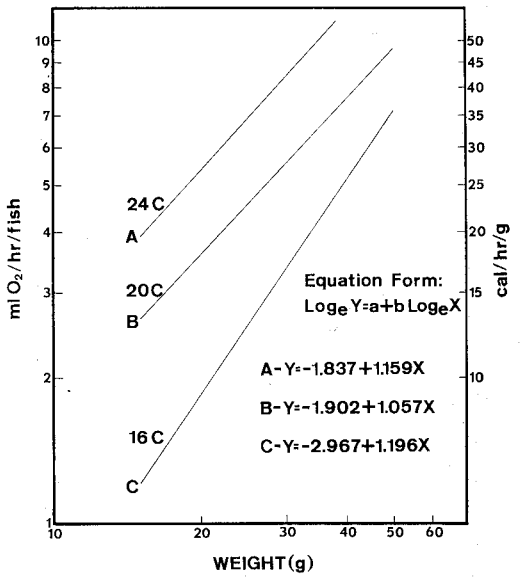

FIGURE 4.—The log-log relationship between oxygen consumption and weight of an entire fish acclimated at three temperatures and one salinity (30 ‰); A-C are expressed in Log_e in the figure; Log_{10} expression for A-C are as follows: Y = oxygen; X = weight.

A $Y = -0.792 + 1.156X$
B $Y = -0.820 + 1.061X$
C $Y = -1.343 + 1.259X$

ous variance ($\chi^2 = 6.81$, 3 df) existed between oxygen levels at each temperature.

The coefficient of variation and mean detectable differences in oxygen uptake levels were inversely related to temperature at 12 C, 16 C, and 20 C (Table 3).

The relation between metabolism and weight calculated for fish acclimated to 30 ‰ salinity is represented by the allometric expression $Y = aX^b$ (Fig. 4). The range in weight did not exceed 46.0 g (Table 2). In all instances, the b values exceeded unity and no significant differences occurred between slopes calculated for the various temperatures. Since the level of metabolism was also directly related to temperature, two factors, temperature and weight, were necessary for calculation of a fish's energy expenditure under routine metabolic conditions. A 30-g fish at 24 C and 30 ‰ salinity, therefore, would require a minimum of 45 calories per hour to maintain its metabolism; the same fish at 16 C would require only about 17 calories per hour.

Oxygen consumed by flounder subjected to lower salinities following acclimation in the chamber was determined (Table 2). Again, variation in uptake stabilized following the 45-minute period at the given salinity; a total time of 3 hours elapsed under test conditions.

TABLE 3.—*Coefficient of variation and mean detectable difference in oxygen consumption (ml O_2/hr/g) of winter flounder for 2 hr 15 min, and 4 hr 30 min periods. Large and small fish at 30 ‰ and at 10 ‰ and 20 ‰ are grouped: S = standard deviation; n = sample size; $\bar{x}$ = mean of oxygen consumption*

Temperature	Coefficient of variation = S/$\bar{x}$		Combined observations	Mean detectable difference = $\sqrt{(2\ St/n).05}$			
				2 hr 15 min Confidence level		4 hr 30 min Confidence level	
	2 hr 15 min	4 hr 30 min		0.05	0.20	0.05	0.20
24 C	19.2%	20.0%	8,6	0.0627	0.0375	0.0498	0.0330
20 C	10.9%	26.2%	7,7	0.0258	0.0152	0.0380	0.0224
16 C	29.9%	31.3%	6,6	0.0497	0.0285	0.0775	0.0445
12 C	84.4%	64.4%	7,7	0.0836	0.0492	0.0127	0.0075

Only values recorded during the two final time periods, 4 hours and 4½ hours, respectively, were analyzed.

Respiration rates at the lower salinities for both time periods, 4 and 4½ hours were calculated for both size groups (Table 2). Again, a simple linear relation between oxygen consumption and temperature gave high correlation coefficients (average r = .90). Differences in rates between the two size groups and between the two salinities within each time period were not significant. Data were pooled for sizes, salinities and times to yield a common regression line for all flounder at lower salinities: $Y = -0.178 + 0.015X$ (Fig. 2). The exponential relation values are: $Y = -7.301 + 0.246X$ (Fig. 3). Application of Bartlett's test of homogeneity of variance between oxygen levels at each temperature for the common line indicated that variances were not significantly different ($\chi^2 = 3.83$, 3 df).

I conducted a test to determine whether the lower oxygen consumption at lower salinities was due to fatigue caused by length of time under test conditions or due to the decrease in salinity. Animals (150–175 mm total length) acclimated at 30 ‰ and 20 C were placed directly in the chamber and subjected to 10 ‰. After 1¾ hours oxygen uptake stabilized within the range 0.050–0.063 ml O_2/hr/g. There was no significant difference between uptake rates of fish held 1¾ hours in the chamber and those retained for 5 hours in the chamber. When salinity was increased to 30 ‰, oxygen consumption increased and all values exceeded 0.063 ml O_2/hr/g after 2 hours at the higher salinity and a total of 4 hours in the chamber. The quantity of oxygen consumed was positively correlated with increasing salinity in Age-1+ winter flounder.

The coefficient of variation was inversely related to temperature for 12 C, 16 C and 20 C at the 4½-hour rates. A high coefficient of variation, 64%, occurred at 12 C. The mean detectable difference was calculated for each temperature (Table 3).

Metabolic rates (ml O_2/hr/fish) were calculated as logarithmic function of increasing weight according to the equation $\text{Log}_{10} Y = a + b \text{ Log}_{10} X$ for each temperature at the reduced salinity. The equations are:

Temperature	Equation
24 C	$Y = -2.927 + 1.1963X$
20 C	$Y = -1.076 + 1.0799X$
16 C	$Y = -2.000 + 1.4572X$

DISCUSSION

Test conditions for determining metabolism of winter flounder were selected after analysis of field data on seasonal conditions in the Weweantic River Estuary, Wareham, Massachusetts. Salinities commonly range from 20 ‰ to 30 ‰ on a tidal cycle during the spring and fall seasons when temperatures are in the 12 C to 16 C range (Topp, 1967; Frame, 1971). Summer temperatures (20–24 C) are generally correlated with higher salinities (up to 30 ‰). Short days characterize the temperate zone spring and fall seasons, and photoperiod interacts with water temperatures. Roberts (1964) found that capacity adaptation in metabolic response to photoperiod length occurs in the pumpkinseed, *Lepomis gibbosus*. He also found that a 3-day acclimation period was not sufficient for full thermal acclimation to a new temperature. All of these variables were considered in these respiration experiments.

Initial variability in oxygen consumption was caused by the slight disturbance of the fish when I attached the chamber to the measuring apparatus. After 1 hour, rates stabilized and variability at the remaining time periods decreased. The extreme variation in oxygen uptake which occurs upon placing fish in a chamber (Keys, 1930; Wells, 1932) was avoided when a specimen was acclimated to the chamber for 24 hours prior to drawing samples.

Determinations of the freezing point depression ($\triangle$ t) of sera from the starry flounder, *Platichthys stellatus*, indicate that a minimum of 10 hours is required for stabilization of respiration rate at a new salinity (Hickman, 1959). In my study, acclimation on the time scale of a tidal change was desired. Others have expressed less variability in respiration rates, but they did not contend with the prob-

lem of changing salinities. Both Hickman (1959) and Edwards et al. (1969) for example, were able to acclimate their test specimens for extended periods to the desired temperatures and salinities.

Respiration rates (ml O_2/hr/g) for fish acclimated to 30 ‰ salinity were not significantly different between the two size groups. This suggests that the metabolic rate (per gram) for larger individuals is not different from smaller fish within the year-class although the data are variable. More determinations at lower temperatures might have shown differences.

The slope of the regression of oxygen consumption on temperature plotted on a semilogarithmic scale (Fig. 3), indicates that the rate decreases at higher temperatures. This phenomenon corresponds to a reduced Q_{10} at increased temperatures (Beamish, 1964). Normally, this interpretation is reserved for description of the metabolism in a fish of a single weight, for example, 100 g fish of a given species (Beamish, 1964; Parvatheswararao, 1965); however, my fish of different weights (within the year-class) exhibit no significant differences in metabolism. Parvatheswararao (1965) has shown that there are considerable differences in the weight specific metabolism (ml O_2/hr/g) of *Etropus maculatus*, and Beamish (1964) points out that slopes differ between species of the same weight. Considering the bioenergetic approach, the average uptake rates per gram may be more meaningful in terms of the age group or population than consideration of different species which deviate from the norm. I suggest that future comparative studies of metabolic rates consider the stage of development of the experimental species.

The relation of body size to metabolism remains a controversial subject among physiologists working with respiration. Winberg (1956) found that under standard conditions, the $\log_{10}$-$\log_{10}$ expression relating metabolism to weight gives constant values which characterize many fish species; the exponent or slope (b-value) was 0.8, and the level of metabolism (a-value) 0.3 (Paloheimo and Dickie, 1966). Prior to this interpretation, three metabolic types had been described by van Bertalanffy (1951):

1. Metabolic rate proportional to surface (b-value of 0.66); fish.
2. Metabolic rate proportional to weight (b-value 1.0); insect larvae.
3. Intermediate rates between the surface and weight proportionalities.

Experimental values for the slope of the log-log relationship generally fall between 0.7 and 0.9 and are, therefore, greater than those originally proposed by van Bertalanffy for fish. Dehnel (1960) derides the validity of such "standard" values, particularly when rate functions vary with various internal and external parameters.

Slopes of the log-log relation between oxygen consumption and weight for fish acclimated to 30 ‰ salinity were all greater than 1.0 This high rate may be related to the small weight range of fish tested (11.25 g to 56.88 g) and suggests that the gills are not limiting the supply of dissolved oxygen to the blood. High b-values have been reported by other investigators. The metabolic rate of the sand dab, *Citharichthys stigmaeus*, is 0.905, although this species rarely exceeds 50 g as an adult (Hickman, 1959). Job (1969) found weight exponents for the cichlid *Tilapia mossambica* as high as 0.999, and Beamish (1964) shows slope values for the brook trout, *Salvelinus fontinalis*, of 1.107, 1.014 and 1.026 at 10, 15, and 20 C, respectively. Zeuthen (1953) reports that metabolic rates undergo significant changes in many animals during their development.

Fish subjected to lower salinities showed a 40–50% decrease in the quantity of oxygen consumed (ml O_2/hr/g) at each temperature, though the rate at which fish consumed oxygen did not differ between the low salinities, 10 ‰ and 20 ‰ combined, and the high salinity 30 ‰. The decrease in respiration represents a significant decrease in energy expenditure but it is not directly proportional to the sea water dilution (10 to 20 ‰). This phenomenon is apparently the result of a reduced osmotic load as the external medium approaches isotonicity. Job (1959) found that 5-g *Tilapia* had the lowest level of consumption in 50% sea water while 80-g fish had the

lowest level in fresh water. I found all fish exhibited a decreased level of metabolism at lower salinities, and there was little evidence supporting size dependent effects on a per unit weight basis.

Observations on the freezing point depression of sera collected from adult *P. americanus* during the winter and summer suggest a seasonal adaptation to salinity changes; estuarine residents (winter fish) have a Δ t 0.63 (Pearcy, 1961). The effect of salinity adaptation was apparently overlooked by Umminger (1970), who contests Pearcy's findings but fails to provide information on size and sex of fish and salinities at the time of capture. Adjustment of the metabolic level by immature fish indicates the euryhaline nature of this species and suggests a physiological reason why young flounder are found in estuaries.

Metabolic rates (ml O_2/hr/fish) of whole fish at lower salinities were slightly greater than the rates of fish acclimated to 30‰ salinity, and the level of metabolism was significantly lower. Several investigators have shown that uptake rates increase at lower salinities (Kinne, 1952; Parvatheswararao, 1965; Job, 1969). They disagree, however, on the physiological mechanisms which cause changes in the weight exponent and metabolic level (Paloheimo and Dickie, 1966). Deviations from the Winberg (1956) weight metabolism relationship ($T = 0.3\ w^{0.8}$) results from at least three factors: (1) reduced weight range of individuals examined; (2) lower salinities; and (3) lack of acclimation to the new salinity. Significant differences in the weight exponent (0.8) have been demonstrated for other estuarine fish (Hoss, 1967).

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